

CHARGE NUMBER: 6902  
PROJECT TITLE: BIOCHEMICAL SPECIAL INVESTIGATIONS  
PERIOD COVERED: February 1-28, 1983  
PROJECT LEADER: T. Yu  
DATE OF REPORT: March 31, 1983

1. V79 SISTER CHROMATID EXCHANGE ASSAY<sup>1</sup>

A four-hour treatment with the direct acting positive control compound ethylmethanesulfonate (EMS) induced a dose related increase in sister chromatid exchanges (SCEs) in V79 cells. SCEs per metaphase chromosome were:  $7.5 \pm 2.0$ ,  $11.3 \pm 2.8$ ,  $19.5 \pm 4.7$ ,  $17.6 \pm 2.1$  and  $27.7 \pm 6.7$  at 0, 0.3, 0.6, 1.0, and 3.0 mM, respectively. No second division cells were observed at the highest dose tested, i.e., 10 mM. A replicate experiment has been conducted and the SCEs are being scored currently. These results will be evaluated to determine interexperimental variations.

2. <sup>32</sup>P-POSTLABELING ASSAY<sup>2</sup>

New shipments of <sup>32</sup>P-ATP and DNA digestion enzymes were received this month. These materials were used for digestion and phosphorylation reactions. As a quality control measure, the <sup>32</sup>P-ATP was chromatographed and autoradiographed (TLC-AR) upon arrival and subsequently at time intervals throughout the month. The TLC-AR results showed that <sup>32</sup>P-ATP contained a major highly radioactive spot and five additional spots. The RF value of the major spot was comparable to that of the authentic ATP. The radioactivity of the additional spots as reflected by the intensity of the spots increased with increasing storage time. Five replicate phosphorylation reactions were carried out using authentic nucleotide monophosphates. The results indicated that the reaction was proceeding and the impurities in <sup>32</sup>P-ATP did not interfere with the reaction. Methods and procedures on quantitation of radioactivity by Cerenkov counting is being worked out in cooperation with Project 2501 personnel.

Two experiments were carried out on the digestion of calf thymus DNA and the subsequent phosphorylation with <sup>32</sup>P-ATP. The digestive enzymes were dialyzed overnight. The results showed that both the digestion and phosphorylation reactions proceeded.

Work will proceed to finish assay establishment by using positive control compounds.

3. NEUROCHEMISTRY (with Project 1610)<sup>3</sup>

Two experiments were carried out in this area.

4. TRADESCANTIA STAMEN HAIR MUTATION ASSAY<sup>4</sup>

A visit was made to Brookhaven National Laboratory to learn the methodology of this assay system. Cuttings and potted plants were brought back and one experiment is currently in progress.

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5. REFERENCES

1. Garcia, H. D. Notebook No. 7722, pp. 94-116.
2. Davies, B. D. Notebook No. 7791, pp. 67-81.
3. Davies, B. D. Notebook No. 7791, pp. 83-95.
4. Yu, T. Notebook No. 7729, p. 105.

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